# TRAINING MANUAL

# POSTHARVEST AND VALUE ADDITION TECHNIQUES IN SEAWEEDS

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**Cochin - 682018**

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**CENTRAL MARINE FISHERIES RESEARCH INSTITUTE**

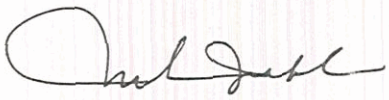
(INDIAN COUNCIL OF AGRICULTURAL RESEARCH)

P.B.NO.1603, COCHIN-682018

## Foreword

Polysaccharides produced by seaweeds form the basis of economically important and expanding industries such as agar, agarose, algin and carrageenan which are used as ingredients in food, pharmaceutical and other industrial and consumer products. Seaweed industries have been slow to realize the fact that these phycocolloids have been prepared with the same specifications and blends from the same raw materials for many years.

Post harvest and value addition techniques are vital needs to the commercial viability of seaweed industries in India. Seaweeds could not earn the name sea-vegetables in India for want of proper value addition techniques. This training is aimed at making a pioneering attempt in this regard. I wish to place on record my appreciation of the task undertaken by the seaweed research team of this Institute to conduct a training on the major aspects of post harvest techniques in seaweeds. I am confident that this training manual will be of help to the trainees during their practical training and in their research endeavours.



(Dr. Mohan Joseph Modayil)
Director

## CONTENT

## 1. Seaweed resources and their distribution in India

*P.Kaladharan and N.Kaliaperumal*

Seaweeds or marine macroalgae consist of taxonomically distinguished groups such as Chlorophyta (green seaweeds), Phaeophyta (brown seaweeds) and Rhodophyta (red seaweeds). They are generally found attached to rocks, pebbles or other aquatic plants in the intertidal or subtidal regions of the sea. seaweeds are the natural source of phycocolloids such as agar-agar, algin and carrageenan. They are also the important source of protein, essential amino acids, fatty acids, vitamins, minerals, iodine, bromine and natural pigments. The amino acid methionine and tryptophan which are reported from seaweed have not been traced in vegetables. A number of tropical seaweeds are eaten directly (sea vegetables), including green algae (*Ulva, Enteromorpha, Monostroma, Caulerpa)* brown seaweeds (*Dictyota, Laminaria, Cladosiphon, Padina)* and red seaweed (*Gracilaria, Porphyra, Eucheuma)* for their minerals, vitamins and low fat content. The major economic significance of seaweeds is however linked with the polysachharide that certain red and brown seaweed species contain. Agar, carrageenan and aligns have all achieved commercial significance because of their food, industrial and biochemical applications.

It had been estimated that seaweed resources of the world comprise about 1460 million tones of brown algae; 261 million tones of red algae and that the total seaweed production may be about $1721 \times 10^4$ tones annually. In India, there are 844 species of marine algae comprising 216 species of Chlorophyta, 191 species of Phaeophyta, 434 species of Rhodophyta and 3 species of Xanthophyta. Out of which 60 species are commercially important.The south east and north west coasts and the Andaman- Nicobar and Laccadive archipelagoes harbour a variety of seaweeds with rich biomass and species diversity. The standing stock of seaweeds in India is estimated to be 2.6 lakh tones ( Table) comprisisng 6 % agarophytes, 8 % carrageenophytes, 16 % alginophytes and the remaining 70 % green and other non commercial seaweeds (Devaraj *et al.*, 1999).

**Estimated Seaweed resources of India** (Chennubhotla,1992)

| Regions | Quantity (tonnes in wet wt.) | References |
|---|---|---|
| Andaman & NicobarIsalnds | 90,939 | Chennubhotla, 1992 |
| Tamil Nadu (Shallow,0-5 m) | 22,044 | Anon, 1979 |
| Tamil Nadu (Deep, 5-22 m) | 75,372 | Anon, 1989 |
| Gujarat | 20,155 | Chauhan & Krishnamurthy, 1968 ; Chauhan & Mairh, 1978 |
| Maharashtra | 20,000 | Untawale et al. 1979 |
| Lakshadweep Islands | 19,345 | Anon., 1989 |
| AndhraPradesh | 7,500 | Anon., 1984 |
| Orissa | 2,521 | Chennubhotla, 1992 |
| Goa | 2,000 | Dhargalkar, 1981 |
| Kerala | 1,000 | Chennubhotla et al., 1988 |
| Total | 2,60876 | Chennubhotla, 1992 |

Luxuriant growth of seaweeds is found in southern coast of Tamilnadu, Gujarat, Lakshadweep and Anadaman-Nicobar Archipelagos. Rich seaweed beds occur at Mumbai, Ratnagiri, Goa, Karwar, Thikodi, Varkala, Vizhinjam, Pulicat and Chilka Lakes. There are about 40 seaweed industries function in India producing algin and agar, depending only on natural resources. These industries do not as yet produce the required quantities of phycocolloids such as agar, algin and carrageenan. As a result, India imports agar, algin and carrageenan every year spending considerable amount of foreign exchange.

## 2. Value addition to *Gracilaria edulis* for improving agar yield and quality

*P.Kaladharan, A. Chandrasekhara Rao* and *J. Ramalingam*

Agar is the major constituent of cell wall polysaccharide of certain red algae, especially the members of the Families Gelidiaceae, Gelidiellaceae and Gracilariaceae. Approximately 60% of the world's present production is derived from *Gracilaria* spp. Generally they yield low quality of agar due to high sulphate content and hence they are called 'agaroids' or 'gracilaria gum'. Quality of agar is the sole criteria for its price, which is decided by gel strength, sulphate content and melting point of agar. The current requirement in the international food market on agar is gel strength equal to or greater than 750 g/ $cm^2$ (1.5% gel) and sulphate content less than 4%. Whereas Japanese grade- 2 agar requires gel strength above 220 g/ $cm^2$ and sulphate content between 1-2 %. *Gracilaria edulis* normally yields agar of gel strength 123 g/ $cm^2$. Hence any value addition to the indigenously produced agar such as sulphate content reduction and gel strength increase not at the expense of yield will definitely make the agar industry economically viable.

**Procedure:**

***Cleaning & drying:***

Wash the thallus of *Gracilaria edulis* in running tap water to remove sand and other epiphytes.

Dry them in sunlight for 2 days until dried completely.

***Extraction:***

Soak the dry seaweeds (20 g) for 11 hours in fresh water.

Transfer them into 2.0 – 3.0 N solution of NaOH. Maintain at 80 ± 2° C.

After one hour wash them with running tap water to remove traces of NaOH.

Decant the water. Transfer them to a beaker and add 400ml distilled water and 40 g $CaCl_2$ to reduce the loss of agar while processing.

Adjust the pH to 6.3 to 6.5

Boil the content in an autoclave at 1 $kg/cm^2$ pressure for 2hr.

Filter the hot extract through a muslin cloth and press in an expeller

The residue was re-extracted with 100 ml hot (85 – 90 ° C)water

Discard the residue and pool together the filtrates, allow to form a gel at room temperature.

Freezing, thawing and finally drying the gel gives the end product.

***Yield:***

Calculate the percentage yield of dry agar as:

$$\text{Yield (\%)} = \frac{\text{Dry weight of agar obtained (in gm)}}{\text{Dry weight of seaweed used (in gm)}} \times 100$$

***Gel strength:***

Make agar solution of 1.5% (wt/v) by weighing 1.5 gm of dry agar and dissolve in 100 ml distilled water with gentle heating in a water bath.

Allow the molten solution to form gel at room temperature for 12 hour

Place the cylindrical plunger (Ø 1 cm) of the gelometer (Fig) just on the surface of the gel

Add weights gradually on the top pan until it breaks the gel in 20 seconds

Note the weight required to break the surface of the gel (1.5%) as gel strength of the agar and express in $gm/cm^2$

***Melting point:***

Keep the agar gel (1.5 %) in hot water bath.

Place the thermometer bulb in the center of the gel and then note the rise in temperature.

Place the spherical glass beads (Ø 3 mm) on the top of the gel.

Note the temperature at which the glass beads start sinking to the bottom as the melting temperature of the agar.

***Sulphate content:***

Reagents: A- Saturated $Mg(NO_3)_2$ in $HNO_3$

B- Glycerol: Ethanol (1:2)

C- solution of NaCl – HCl

Weigh accurately 100 mg dry agar samples in the crucible.

To this add 2.0 ml of reagent- A and allow the reagent to evaporate in the hood.

After complete evaporation of fumes, place the crucibles in the muffle furnace at 400° C for 5 hrs.

Cool them to 80 ° C after 5 hrs add 5 ml of 1.0 N HCl to each sample

Filter the solution through No.1 filterpaper (Whatman).

Make up the filtrate to 50 ml and then transfer the content to 150 ml beaker

Add 0.5 ml of reagent C and 10 ml of reagent B.

Stir the mixture and while stirring add 0.2 gm of $BaCl_2$ to dissolve.

Measure the OD at 425 nm through a spectrophotometer

Prepare a standard sulphate by dissolving 0.1479 gm of anhydrous sodium sulphate in one liter distilled water

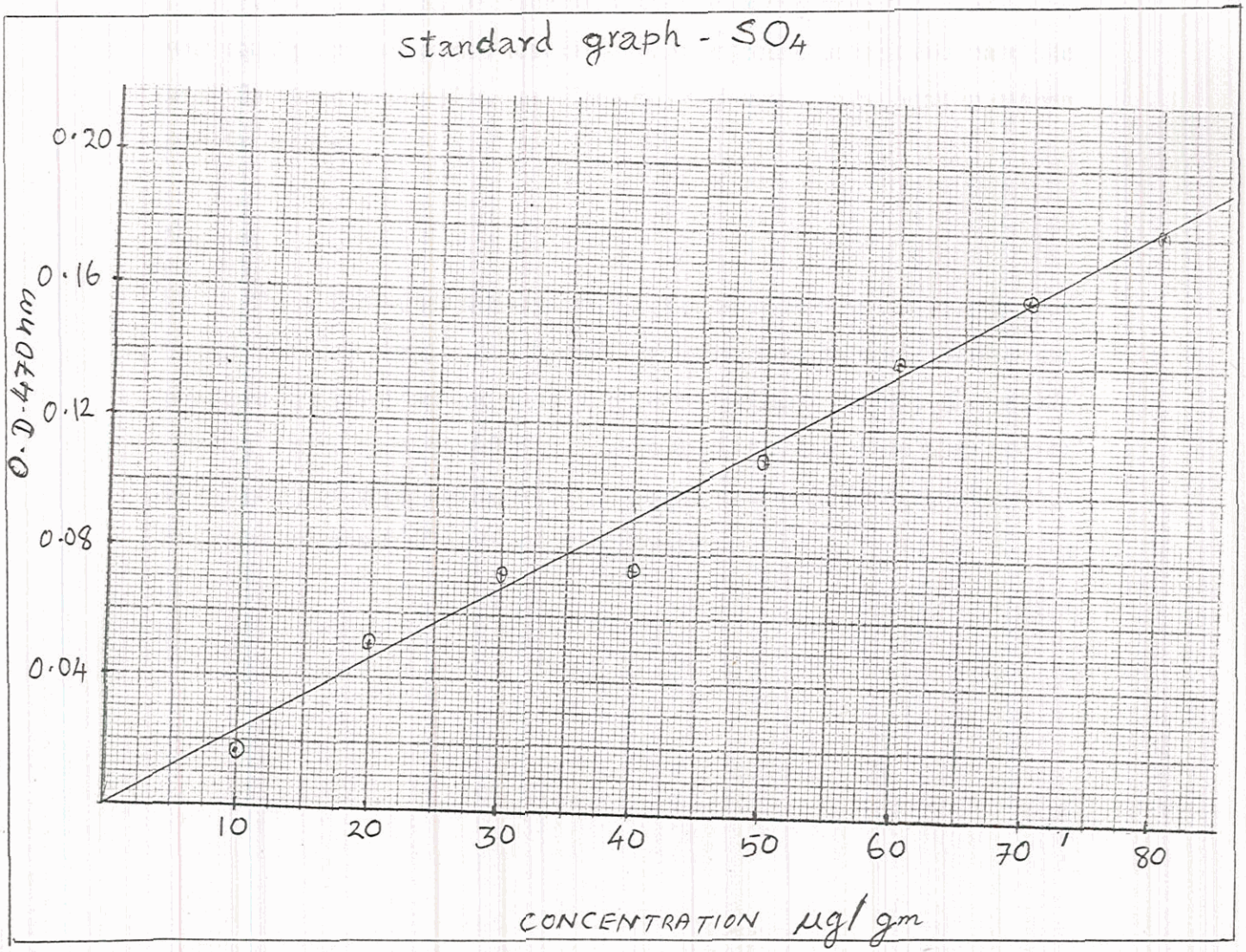
Prepare aliquots in the range of 10-70 µg. Measure the OD and prepare the standard graph.

**Result**

| NaOH (N) | Yield (%) | Gel strength ($g/cm^2$) | Sulphate content (%) | Melting point (° c) |
|---|---|---|---|---|
| 0.0 (control) | | | | |
| 0.5 | | | | |
| 1.0 | | | | |
| 2.0 | | | | |
| 3.0 | | | | |

**European and American standards for bacteriological grade Agar**

| *Specifications* | *European std* | *American std* |
|---|---|---|
| Moisture | <10% | <10% |
| Ash | <4.5% | <6.5% |
| Gel strength | 800 –1100 g/cm$^2$ | 800 –1100 g/cm$^2$ |
| PH(1.5% before autoclave) | 7.0 ± 0.4 | 7.0 ± 0.4 |
| Melting point (1.5%) | 85 ± 5 ° C | 85 ± 5 ° C |
| Gel point (1.5%) | 35 ± 3 ° C | 35 ± 3 ° C |
| Clarity (1.5%) | <12 NTU | <12 NTU |
| Colorimetry Abs 430 nm) | <0.200 | <0.200 |
| Particle size | 95% pass mesh | 60 ASTM |



## 3. Fuel and Manure from Agar Factory Discharge

*P.Kaladharan*

Utilization of seaweeds for commercial purposes was started in India since three decades ago. Coastal farmers with ready access to sea have used seaweeds as green manure. Compared to the popular brand of farmyard manure, seaweeds are known to contain similar nitrogen values, about one third as much as phosphate and about three times as much as potassium. Polysaccharide yield from Indian agarophytes using the existing method of extraction is not more than 15% and the daily discharge of 80 – 85% is being piled up in the factories itself in huge quantities unutilized. This unutilized algin/ agar factory discharge (AFD) can be utilized as fuel for cooking and as manure for crops.

**Fuel:**

"Fuel cakes" can be made from AFD by mixing fresh AFD (paste like) with equal proportion of rice husk or saw dust or groundnut husk and made into small flat cakes and dried in sun. The dried fuel cakes can be burnt in earthen oven for cooking.

**Manure**

The AFD obtained from agar factories or algin factories can be utilized as manure for crop plants. The dry AFD collected from seaweed factories are powdered in a pulverizer and 20 to 30 g can be applied to the base of vegetable crops as a basal manure. After AFD application, the base of the plant may be forked gently to ensure mixing with soil. Application of AFD to vegetable crops had improved growth of plants and early flowering.

*Procedure*

Take 10 equal sized pots.

Fill them with garden soil three fourth

Sow two seeds of cowpea in each pot

To five pots add 30 gm of AFD powder and gently fork the base to mix well. Another set of five pots serve as control (without AFD)

Add 100 ml of water/pot twice a day to avoid leaching

Observe the growth, number of leaves, shoot length etc and record. Compare the difference in the growth between AFD treated and control pots.

## 4. Extraction of Carrageenan

*P. Kaladharan*

Some 600 years ago the coastal residents of Carragheen county on the south Ireland first used the seaweed Irishmoss botanically known as *Chondrus crispus* and noted the colloid that can react with milk. Later this polysaccharide was named as carrageenan. Carrageenans are aromatic polyelectrolytes and have structural feature of being linear polysaccharide built up of alternating 1,3 linked β-D-galactopyranosyl and 1-4 linked α -D-galactopyranosyl units. Carrageenan can be extracted from *Hypnea* or from *Kappaphycus.*

**Procedure**

Before extraction the seaweed is pulverized, bleached and dried. Pulverizing improves the yield and bleaching improves the quality of carrageenan.

***Pulverization***

Grind the dry weed in mechanical pulverizer and then sieve the powder through 40-50 μ mesh sieve.

***Bleaching***

Treat pulverized seaweed with five volumes of acetone

Filter to get rid of green liquid containing pigments.

Treat the residue with double the volumes of diethyl ether with stirring which ensures further bleaching.

Filter and dry the residue at 60°c.

***Extraction:*** (Sodium bicarbonate method)

Treat 5 gm of dried seaweed powder with 100 ml of 0.5 M sodium bicarbonate solution .

Autoclave for 1½ hrs.

Filter the viscous solution while hot, into a 500 ml beaker

Cool the extract to room temperature

Observe if the extract becomes gel immediately on cooling.

If the extract does not become gel on cooling but remains highly viscous, add 2.5 times the volume of alcohol.

Remove carrageenan by filtration and dry in an oven at 60 ° C for overnight.

If gel is formed, keep it in a freezer, thaw the frozen gel and then dry

Weigh the dry carrageenan obtained and calculate the yield.

***Yield:*** Calculate the yield of carrageenan as same as agar.

## 5. Fodder from seaweeds

*P.Kaladharan*

Livestock wealth of India comprising 204 million cattle (15 % of world production), 84 million buffaloes (53% of world) and 118 million goats (20% of the world) is in a very critical state in the face of mounting deficiency of feed and fodder (*Indian Livestock Review*, 1999). As per 1995-96 data (FAO 1996), availability of oil cake was only 20.74 million tonnes (mt) as against the annual requirement of 496.58 mt. Similarly the availability of green and dry fodder was 300 mt and 310 mt respectively as against the requirement of 2037 mt and 722 mt respectively.

Shrinkage of cultivable land due to urbanization and shortage of water limit the possibility of producing more feed and fodder to livestock from land. Sea remains untapped and the seaweed resources have got immense potential to fill the gap in India. Seaweeds were used as animal feed as early as first century BC by the Greeks. Seaweed has been used by farmers living near the sea in Europe. In Norway *Ascophyllum* is used as pigmeal. *Rhodymenia palmata* a red seaweed is called cow weed in Brittany and horse weed in Norway. Dried and processed seaweeds have been used as animal feed in Europe and North America.

Seaweeds are rich in protein (20- 25%), carbohydrate (50-70%), vitamins, minerals and certain drugs. When used in animal feed, cows produced more milk, chicken eggs became better pigmented and horses and pets became healthier (White and Keleshian, 1994). Presence of tocopherol and Vitamin E in seaweeds did increase the fertility rate and birth rate of animals when used as fodder. Both milk production and fat content have been found to increase by using seaweed as part of the diet. Feed supplemented with *Gracilaria* and /or *Spirulina* to layer chicks (white leghorn) increased the number of eggs, size and colour of yolk (Chaturvedi *et al.* 1985). Dave *et al* (1977) assessed the possibility of seaweeds being used as supplementary animal feed and they reviewed the feeding trials of

farm animals with seaweeds conducted in Japan, Germany, the UK and Norway. Cattle grazed on *Laminaria* sp. Based diet have better natural resistance to diseases such as foot and mouth. In the USA, when hens were fed with 1.25% seaweed added to their normal ration, the proportion of thin celled-eggs were reduced from 3-19% and when after 3 months the seaweeds addition to the diet was discontinued, the proportion of thin celled-eggs again increased.

Experiments using 3500 sheep showed that an addition of 35 gm per day of seaweed meal gave a 3.3% increase in winter wool which was increased a further of 17% even if the sheep had no mineral supplement In the case of cows, use of seaweed meal increased butter fat content by 6.8% over a seven year experimental period and also reduced the incidence of mastitis. Seaweeds are known to have essential aminoacids ratio, which is considered optimum for human food. Studied indicate that digestibility of *Macrocystis pyrifera* and *Sargassum* spp by bovine cattle is 85% and 55% respectively. Seaweed treated pasture forages increased immunity in pigs and chicks.

India is endowed with 6000km coastline and bestowed with more than 0.2 mt/year wet harvestable biomass of seaweeds belonging to 700 species. Of these nearly 60 species to the tune of 30 % are economically important for their polysaccharides. Others amounting to 70 % of the biomass are underutilized. These underutilized or unutilized seaweed resources can be used as fodder or feed for cattle either raw or processed. Species of *Enteromorpha, Ulva, sargassum, Chnoospora, Acanthophora, Hypnea, Gracilaria, Chaetomorpha* and *Caulerpa* can best be tried as fodder. These can be utilized as silages prepared with paddy straw when available in glut conditions and silages can be stored.

## 6. Cytokinins from marine green alga *Caulerpa racemosa*

*P. Kaladharan*

Among the chemicals isolated from algal sources, the common ones are sesquiterpenoids from *Dictyopteris divaricata* (Phaeophyceae) and *Laurencia glandulifera* (Rhodophyceae), friedelin from *Monostroma nitidum* (Cyanophyceae) and sterols and acyl glycerol from *Colpomenia perigrina* and *Scytosiphon iomentaria* (Phaeophyceae). Calerpicin, an indole derivative and Caulerpin, a pyrazine derivative from *Caulerpa racemosa* and *C. lamoureuxii* and Cytokinin from *Ascophyllum nodosum* are some of the major examples for bioactive substances obtained from green seaweeds (Chlorophyceae). Procedure for extraction of Cytokinin from *Caulerpa racemosa* is shown below (Kaladharan and Sridhar, 1999).

**Procedure**

Grind fresh *Caulerpa racemosa* (1 Kg) with 400 ml distilled water.

Add 1.6 l of ethanol to the mixture to make the final concentration of ethanol 80% (v/v).

Filter the mixture through filterpaper No.1 (Whatman)

Remove the pigments from the filtrate with petroleum ether (100 ml twice) taken in a separating funnel.

Adjust the pH of the pigment free extract to 2.5 with HCl.

Allow the extract to percolate through a column of CM-cellulose at the rate of 20 ml/hr.

Wash the column after complete percolation with 200 ml of 80 % ethanol and then with double distilled water.

Discard the wash

Elute the Cytokinins adsorbed by the cellulose with 100 ml of 5 N ammonium hydroxide solution.

Concentrate and dry the eluates in a rotary evaporator.

**Assay of Cytokinin activity**

Prepare 1-5 ppm concentration Cytokinin thus extracted from *Caulerpa racemosa* in double distilled water.

Soak etiolated cucumber cotyledons (germinate the seeds in dark) 5 nos in a petri plate each concentration with 2 or 3 replicates and suitable controls (0 ppm) in dark room with safe light.

Maintain this set up in dark

Observe the pigment synthesis in dark after 2 days and compare the levels of chlorophyll synthesis in each concentration.

**Results**

| Conc. of Cytokinin (ppm) | Initial level of total chl.in etiolated cotyledons (µg/g wet wt) | Final level of total chl. synthesized in dark by etiolated cots soaked with cytokinin (µg/g wet wt) |
|---|---|---|
| 0.0 (control) | | |
| 1.0 | | |
| 2.0 | | |
| 3.0 | | |
| 4.0 | | |
| 5.0 | | |

## 7. GABA from *Hypnea valentiae*

*P. Kaladharan*

*Hypnea* a red seaweed is known to contain Heparin. GABA is the popular name for gamma amino butyric acid which is used in the treatment of epilepsy, hypertension and anxiety. GABA induces behavioral and developmental metamorphosis in planktonic molluscan larvae.

**Procedure**

Homogenize 500 gm fresh *Hypnea valentiae* with equal volume of chloroform- methanol solution (1:3).

Centrifuge the mixture at 7000 rpm for 10 min.

Separate the dissolved pigments with petroleum ether as the upper layer when taken in a separating funnel.

Lower layer contains crude GABA, Concentrate and dry in a rotary evaporator.

**Assay of GABA activity**

Dissolve the dry GABA in ethanol and make 1.0 l each of 0, 5, 10, 15 and 20 ppm crude GABA with 30 ppt seawater.

Keep 150 each of pediveliger mussel or oyster larvae in different concentrations of crude GABA prepared.

Observe the settlement of larvae along the sides of the beaker after 48 hrs and compare the rate of settlement with the control (0 ppm).

**Result**

| Time (hrs) | Rate of larval settlement ppm 0 | 5 | 10 | 15 | 20 |
|---|---|---|---|---|---|
| 0 | | | | | |
| 6 | | | | | |
| 12 | | | | | |
| 18 | | | | | |
| 24 | | | | | |
| 36 | | | | | |
| 48 | | | | | |

## 8. Seaweed Liquid Fertilizer

*P. Kaladharan* and *N. Kaliaperumal*

Seaweed extract is made into mineral rich seaweed liquid fertilizer (SLF) and marketed under various trade names. Studies have proved that extracts of *Sargassum*, *Ulva*, and *Spatoglossum* spat 1% strength show favourable response on the germination, seedling vigour, fruit setting and on weight of fruit in crops like groundnut, maize, gingelly., tomato and ber. Seaweed extract are used in hydroponics and for the mass culture of phytoplankton. Seaweed liquid fertilizer was first patented in the year 1912. Another patent in 1962 was offered to Maxicrop Ltd and marketed as "Maxicrop" and "Bio extract",. "Marinure", "SM-3" and "Trident" are the popular brands of SLF in the UK. In India SPIC Ltd is manufacturing and marketing SLF in the name "Cytozyme".

**Procedure**

Wash the seaweed to remove dirt and sand

Dry and then pulverize in a grinder

Boil the seaweed powder with water in the ratio of 1: 10for 2 hrs.

Filter and centrifuge the extract.

The semi-viscous filtrate is used as SLF.

Dry the filtrate at 65- 70 °C to get dry solid, powder and pack it in air tight containers.

This powder can be used as SLF by making 0.5% to 1.5% (w/v) solution with water either as a foliar spray to the canopy or to the base of horticultural crops.

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